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Electron Transfer Of Redox Enzymes To Inorganic Electrodes

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**13. ABSTRACT** *(Maximum 200 Words)*

This project was to develop a protein and peptide-based nanowire and nanoswitch with defined length and properties for a broad spectrum of application in nanobiotechnology. These nanowires were used to develop novel enzyme/conductive matrix in which the activity of redox enzymes could be controlled by direct electron transfer to inorganic electrodes. We investigated synthetic peptide structures as linking agents between target enzymes and conductive electrodes. Directly couple nanocrystals to amino acids, peptide and proteins. This was carried through several methods. One was to couple the nanocrystals to peptides and proteins directly. But the yield was very low and become prohibitively expensive. The alternative was to first couple the nanocrystals to amino acids and through chemical synthesis to make peptides and proteins. We have successfully achieved it.

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One of the key areas is that the coupling nanocrystal to peptides and proteins needs to be mono-functionalization amino acids, namely, one nanocrystal only couples to a single amino acid. This is to ensure the single nanocrystal incorporation into the desired peptide and protein locations. Our observation of dimmer formations, after chemical couple the amino acids, demonstrates the feasibility that we can indeed covalently couple gold nanocrystals to amino acid. However, the yield is low at this moment. Additional effort is clearly needed. We have succeeded in directly and covalently coupled gold nanocrystal to amino acid lysine on the resin. The coupled lysine is then reacted to form a dimmer for visualization. There are high % dimmers in the preparation, suggesting the formation of a mono-functionalized gold nanocrystal with a single lysine.

We have also couple photosyntehsis system I (PSI) onto metal surfaces and kept their activities for extended time. Preliminary results suggest that this coupled PSI is shown to produce both current and voltage. This is in collaboration with Dr. Marc Baldo of MIT.

**Redox protein production and Assay**

We expressed cytochrome c oxidase (CcO) in the bacterial strains. The overexpression strains of Rhodobacter sphaeroides were grown at 30 □C in flasks containing Sistrom's minimal medium. The purified complex was mixed with glycerol (20% (w/v)) prior to freezing, frozen in liquid nitrogen and stored at –80 □C until used.

The activity of CcO is defined in terms of the first-order velocity constant k. k is obtained from the absorbancy decrease in the first minute or from the slope of ln (absorbancy) – time. The specific activity (S.A.) is calculated from the known concentration of Cc and CcO in the assay mixture and the estimated first-order velocity constant k. S.A. = k(Cc concentration)/(CcO concentration).

**Immobilization of CcO to Carbon Particles**

Put the oxidized carbon particles in a flask. Add 0.1 M EDC. To this add 10 mg/ml Ni-NTA or 1mg/ml CcO. Gently mix overnight. After this time, collect the carbon particles by vacuum filtration through filter paper. Wash with PBS.

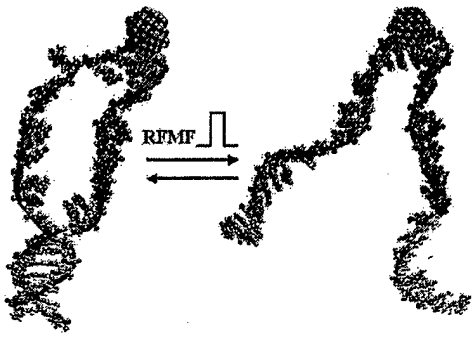
Cytochrome c oxidase was immobilized onto several types of surfaces. There are five principal methods for immobilization of enzymes: adsorption, covalent binding, entrapment, encapsulation, and cross-linking. Immobilization by adsorption is the simplest method and involves reversible surface interactions between enzyme and support material. The forces involved are mostly electrostatic, such as van der Waals forces, ionic and hydrogen bonding interactions, sometimes hydrophobic bonding can be significant.

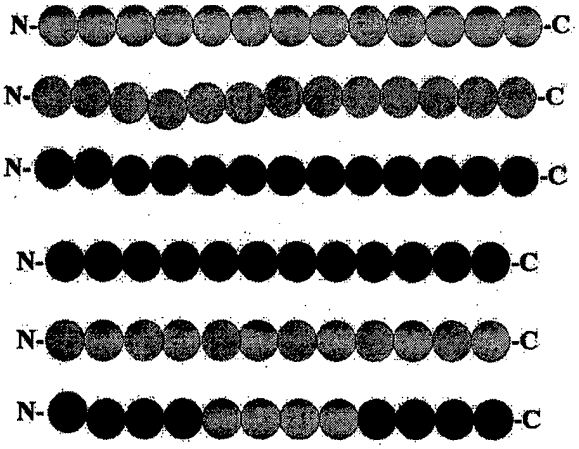
Covalent binding involves the formation of a covalent bond between the enzyme and the support material. The bond is normally formed between functional groups present on the surface of the support and functional groups belonging to amino acid residues on the surface of the enzyme. A number of amino acid functional groups are suitable for participation in covalent bond formation. Those that are most often involved are the amino group (NH2) of lysine or arginine, the carboxyl group (COOH) of aspartic acid or glutamic acid, the hydroxyl group (OH) of serine or threonine, and the sulfydryl group (SH) of cysteine.

Ni-NTA was coupled to gold surfaces. Histidine-Tag linked cytochrome c oxidase were oriental immobilized on gold surface. Substrate cytochrome c concentration effect: the current output increases with the substrate cytochrome c concentration until around 10 uM.

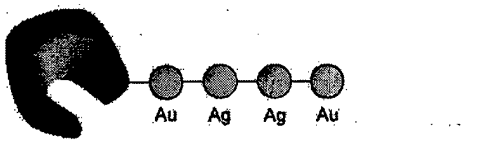
The activity and current of immobilized cytochrome c oxidase on gold surface were studied. In the range of pH 6.5 – 8.5, the immobilized enzyme has highest activity on gold surface. The stabilities of the activity and current output of the immobilized cytochrome c oxidase on gold surfaces were studied. The results show that the oriented immobilized enzymes are more stable and have higher activity than the non-oriented immobilized enzymes.

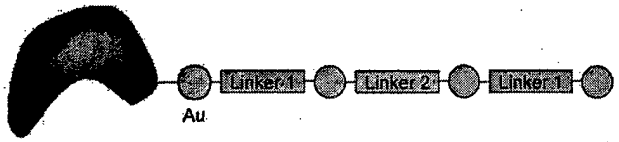
Shuguang Zhang, Ph.D., Bioengineering & Biology and Joseph Jacobson, Ph.D., Physics & Nanotechnology, are two Co-PIs. They direct the overall project. Zhang's group is focused on redox protein purifications, assays and covalently and orientedly link to metal surfaces. Jacobson's group is focused on functionalize nanocrystals to produce nanowires.
Shuwang An, Ph.D., Physical Chemistry, Andrea Lomander, Ph.D., Chemical Engineering & Bioengineering, Xiaojun Zhao, Ph.D., Protein Chemistry & Biochemistry, Patrick Kiley, Biology & Biomedical Enginering MIT '03.




# Multichannel Switches

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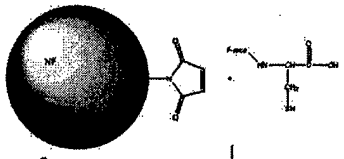
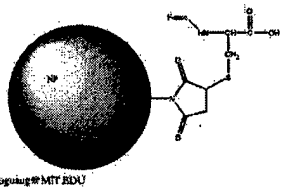


**Metal nanocrystal couples to a single amino acid Cysteine through the -SH bond.**



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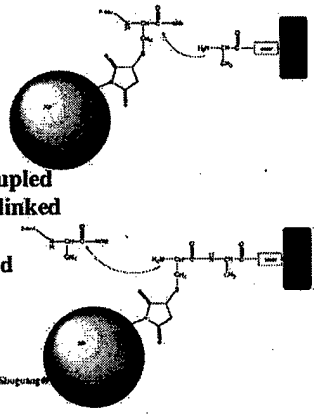
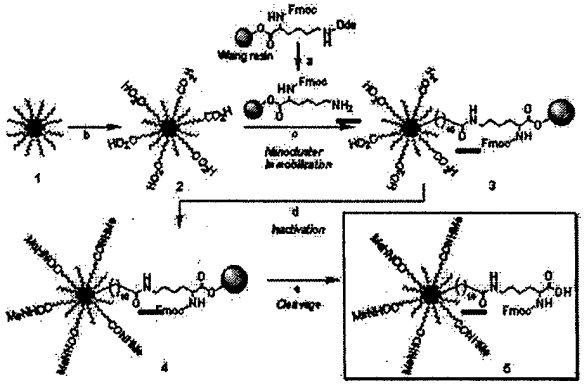
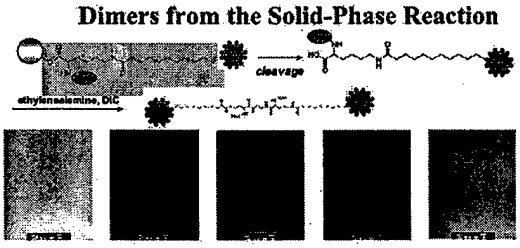


**Metal nanocrystal coupled Cysteine can then be linked to a growing peptide chain using a standard peptide synthesizer.**

**Metal nanocrystal couples to a single amino acid: Lysine linked Wang resin for a single linkage**




**Dimers from the Solid-Phase Reaction**




**Direct synthesis of nanocrystal coupled amino acid. The dimer formation indicates the mono-functionalized nanocrystals, namely, one nanocrystal is coupled to only one amino acid.**

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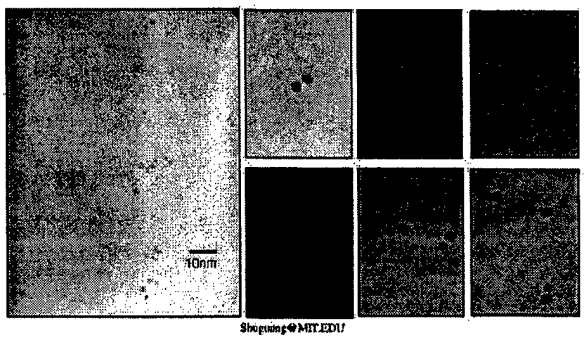
**The bottleneck is to increase the yield of mono-functionalized amino acids**

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